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     (FILE 'HOME' ENTERED AT 14:57:52 ON 09 OCT 2003)

     FILE 'MEDLINE' ENTERED AT 14:58:04 ON 09 OCT 2003
L1          3746 S SKIN (L) DERMAL (L) EPI?
L2           431 S L1 AND MATRIX
L3           220 S L2 AND COLLAGEN
L4             2 S L3 AND (IMPLANT OR DEVICE)
L5            45 S L3 AND ARTIFICIAL
L6            45 FOCUS L5 1-
                 E BOYCE STEVEN?/AU
L7             7 S E1
L8            30 S L3 AND SUBSTITU?
L9        139077 S DERM?
L10        84607 S EPIDERM?
L11        27559 S SKIN (L) (ARTIFICIAL OR GRAFT OR SUNSTITU? OR CULTUR?)
L12         1333 S L9 (L) L10 (L) L11
L13            0 S L12 AND COLLGEN?
L14          306 S L12 AND COLLAGEN?
L15          174 S L14 AND (MATRIX? OR LAYER? OR SCAFOLD?)
L16          174 FOCUS L15 1-

     FILE 'MEDLINE, CANCERLIT, SCISEARCH, CAPLUS, MEDICONF' ENTERED AT
     15:37:01 ON 09 OCT 2003
L17          494 S L15
L18          270 DUP REM L17 (224 DUPLICATES REMOVED)

=> s l18 and gene?
L19           62 L18 AND GENE?
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L8 ANSWER 28 OF 30 MEDLINE on STN
AN 90300729 MEDLINE
TI In vitro effects of **matrix** peptides on a cultured **dermal -epidermal skin substitute**.
SO JOURNAL OF SURGICAL RESEARCH, (1990 Jun) 48 (6) 528-33.
Journal code: 0376340. ISSN: 0022-4804.
AU Cooper M L; Hansbrough J F; Foreman T J
AB Composite **dermal-epidermal skin substitutes** rely on a firm attachment of human keratinocytes (HK) to the **dermal** substrate for graft survival on the wound. An in vitro study was performed assessing whether the addition of **matrix** peptides to the **dermal** substrate effected the **epithelial** thickness. Cultured grafts were made by attaching HK to the external surface of a **collagen**-chondroitin 6-sulfate (GAG) membrane and inoculating human fibroblasts (HF) internally. If the **matrix** peptide (RGD Peptide) was added to the **collagen**-GAG membrane prior to placement of the HK and HF, the resultant **epithelial** layer at the end of the normal 4-day culture period was significantly thicker (19.7 +/- 0.9 microns versus 13.5 +/- 1.0 microns). Subjectively, the HF content was also greater on the peptide-treated grafts. When HF were not placed on the cultured graft, i.e., only **collagen**-GAG membrane, RGD peptide, and HK, the resultant **epithelial** thickness was even greater (28.3 +/- 1.0 microns). These data suggest that addition of **matrix** peptides, which increase cell attachment to the **dermal** substrate, may prove effective in the improvement of this cultured composite **dermal-epidermal skin substitute**.

L8 ANSWER 27 OF 30 MEDLINE on STN
AN 91169069 MEDLINE
TI In vitro and post-transplantation differentiation of human keratinocytes grown on the human type IV **collagen** film of a bilayered dermal **substitute**.
SO EXPERIMENTAL CELL RESEARCH, (1991 Apr) 193 (2) 310-9.
Journal code: 0373226. ISSN: 0014-4827.
AU Tinois E; Tiollier J; Gaucherand M; Dumas H; Tardy M; Thivolet J
AB Using human type IV and type I + III **collagens** and a new, nontoxic cross-linking procedure, we have developed a cell-free bilayered human **dermal substitute** for organotypic culture and transplantation of human **skin** keratinocytes. We have studied the formation of the basement membrane, and the differentiation of keratinocytes grown on the type IV **collagen** layer of this **dermal substitute**, in vitro and after grafting onto nude mice. These studies demonstrated the formation of essential constituents of the basement membrane in culture: hemidesmosomes and deposition of extracellular **matrix** on the top of the type IV **collagen** were observed as early as 6 days after plating of human keratinocytes. Although the keratinocytes formed a well-organized multilayered **epithelium**, they exhibited limited differentiation when grown submerged in liquid medium. However, the multilayered sheet obtained after 14 days in submerged culture was composed of a regular basal cell layer, several nucleated suprabasal cell layers containing granular cells, and several dense, anucleated cell layers. The grafting experiments have shown a good biocompatibility of the **dermal substitute** . It is repopulated by fibroblasts, newly synthesized **collagen**, vessels, and a few mononuclear cells. At Day 14 after grafting, the type IV **collagen** layer was still present and very dense, and the basement membrane appeared as in culture, with numerous well-structured hemidesmosomes and deposition of extracellular **matrix** resembling lamina densa. At Day 55 after transplantation, even if the **epidermal** graft did not exhibit all the characteristics of the normal **epidermis** in vivo, it was very close to it. At this stage, the basement membrane was complete, with structures clearly indicative of anchoring fibrils. This new **dermal substitute** offers many advantages. It is stable and easy to handle. Its production is standardized. The oxidation induced by periodic acid led to a nontoxic cross-linked **matrix**. This **dermal substitute** is the first one entirely composed of human **collagens**. The type I + III **collagen** underlayer is reorganized when grafted. It supports a type IV **collagen** top layer which offers an excellent substrate for keratinocytes, favors their anchorage, and favors the formation of the basement membrane in vitro. This **dermal substitute** could be useful for wound coverage or as an in vitro model for toxicological and pharmacological studies.

L8 ANSWER 24 OF 30 MEDLINE on STN
AN 94064754 MEDLINE
TI Composite grafts of human keratinocytes grown on a polyglactin mesh-cultured fibroblast dermal **substitute** function as a bilayer skin replacement in full-thickness wounds on athymic mice.
SO JOURNAL OF BURN CARE AND REHABILITATION, (1993 Sep-Oct) 14 (5) 485-94. Journal code: 8110188. ISSN: 0273-8481.
AU Hansbrough J F; Morgan J L; Greenleaf G E; Bartel R
AB We have developed and tested in athymic mice a new, cultured, **dermal-epidermal** graft composed of two human cell types coupled with a biodegradable **dermal** scaffold. Cultured, proliferating human keratinocytes (HK) were applied to the surface of a living **dermal** tissue replacement that is composed of human fibroblasts cultured on a polyglactin mesh. After 4 to 6 days of coculture, proliferating HKs achieved confluency on the surface of the living **dermal** tissue replacement. Grafts were then transferred to full-thickness wounds on the dorsum of athymic mice. Sixteen animals were grafted, and the mean percentage of graft take (original wound area covered) on day 20 after grafting was 51.25%. Staining with antibody specific for human involucrin confirmed the presence of HKs on closed wounds, and staining with antibody specific for human laminin revealed a continuous layer of laminin at the **dermal-epidermal** junction on day 20. Animals closed with living **dermal** tissue replacement alone markedly contracted, whereas application of living **dermal** tissue replacement-HK grafts appeared to retard contraction. Because polyglactin mesh fibers are absorbed by hydrolysis rather than by enzymatic degradation, this living composite graft may be more resistant to destruction when placed on excised human wounds than are composite grafts, which are composed of a **collagen matrix**. The inclusion of the living **dermal substitute** may ultimately provide better **skin** quality than is achieved from the use of cultured keratinocytes alone. Fragility of the **epidermal** layer is probably due to the short-term culture of HKs on the living **dermal** tissue replacement, and further efforts to develop a thicker **epithelial** layer may improve graft durability.

L8 ANSWER 23 OF 30 MEDLINE on STN
AN 94190088 MEDLINE
TI Ultrastructural and immunohistochemical characterization of basal cells in three-dimensional culture models of the skin.
SO ARCHIVES OF DERMATOLOGICAL RESEARCH, (1994) 286 (1) 53-61.
Journal code: 8000462. ISSN: 0340-3696.
AU Horiguchi Y; Maruguchi T; Maruguchi Y; Suzuki S; Fine J D; Leigh I M; Yoshiki T; Ueda M; Toda K I; Isshiki N; +
AB Keratinocytes were cultured on fibroblast-free **dermal substitutes** made of type I **collagen** film (**collagen dermal substitute**) and an extracellular **matrix** gel film (**matrix dermal substitute**), each of which was laid on a lyophilized type I **collagen** sponge. The morphology of the basal keratinocytes in these three-dimensional culture models of the **skin** was studied ultrastructurally and immunohistochemically to assess their differentiation to basal cells. The basal keratinocytes in the artificial **epidermis** cultured on the **collagen dermal substitute** showed poorly organized tonofibril networks and desmosomes. Neither the tonofibril-hemidesmosome complex nor the lamina densa were detected along the interface, where many cytoplasmic projections of basal keratinocytes were noted. There were no detectable antigens of type IV or VII **collagen**, LDA-1, or laminin in the interface. Bullous pemphigoid (BP) and 1-2B7B antigens and integrins were expressed along the cytoplasmic membrane and the projections of the basal keratinocytes. A high molecular weight keratin (keratin 1, 68 kDa, 34 beta B4) was detected only in part of the uppermost layers of this artificial **epidermis**. In contrast, basal keratinocytes in the artificial **epidermis** on the **matrix dermal substitute** developed tonofibril networks radiating to desmosomes and hemidesmosomes, under which a primitive lamina densa was present. Basement membrane zone antigens, such as type IV and VII **collagens**, LDA-1 and laminin were noted along the interface as were 1-2B7B and BP antigens and integrins. Laminin and type VII **collagen** were also detected along or in the membrane of the endoplasmic reticulum of basal keratinocytes.(ABSTRACT TRUNCATED AT 250 WORDS)

8 ANSWER 21 OF 30 MEDLINE on STN
AN 96106978 MEDLINE
TI Evaluation of an allogeneic cultured dermal **substitute** composed of fibroblasts within a spongy **collagen matrix** as a wound dressing.
SO SCANDINAVIAN JOURNAL OF PLASTIC AND RECONSTRUCTIVE SURGERY AND HAND SURGERY, (1995 Sep) 29 (3) 211-9.
Journal code: 8707869. ISSN: 0284-4311.
AU Yamada N; Shioya N; Kuroyanagi Y
AB The purpose of this study was to examine whether **epithelialisation** is promoted when an allogeneic cultured **dermal substitute** is used as a biological wound dressing. The **dermal substitute** was prepared by plating fibroblasts on to a spongy **collagen matrix**, and then culturing them for 7 to 10 days. A new animal wound model was designed to measure re-**epithelialisation** quantitatively. A full thickness **skin** defect was made on the dorsum of each of 33 rats; the **skin** was excised, leaving a layer of pannicular carnosus with an island of intact **skin** in the central portion of the **skin** defect. In the first group of rats (n = 13), a piece of cultured **dermal substitute** was applied to the wound, and a medicated covering material was placed over it. Re-**epithelialisation** from the island of intact **skin** was monitored over a period of 7 days. In the second group of rats (n = 10), the wound was covered with an acellular **collagen matrix** in conjunction with the medicated covering material, and in the third group of rats (n = 10), the wound was covered with the medicated covering material alone. Both the macroscopic and histological findings indicated that the **epithelial** migration of the first group of rats was far more rapid than that in the other two groups. It comes to the conclusion that the application of this new fibroblastic cultured **dermal substitute** provided a good environment for the promotion of wound healing.

L8 ANSWER 12 OF 30 MEDLINE on STN
AN 1999379064 MEDLINE
TI Clinical evaluation of an allogeneic cultured dermal **substitute** composed of fibroblasts within a spongy **collagen matrix**
.
SO SCANDINAVIAN JOURNAL OF PLASTIC AND RECONSTRUCTIVE SURGERY AND HAND SURGERY, (1999 Jun) 33 (2) 147-54.
Journal code: 8707869. ISSN: 0284-4311.
AU Yamada N; Uchinuma E; Kuroyanagi Y
AB We have developed an allogeneic cultured **dermal substitute** (CDS) that was prepared by plating fibroblasts on to a spongy **collagen matrix** and culturing them for 7 to 10 days. The **matrix** was freeze-dried from a 1% aqueous solution of bovine-hide atelocollagen. This study was designed to evaluate the efficacy of promoting **epithelialisation** clinically on 26 donor-site wounds for split-thickness **skin** grafts. One half of a wound was covered with an allogeneic CDS and the other half side was covered with a commercially-available freeze-dried porcine dermis (FPD). Both macroscopically and histologically the **epithelialisation** on the area of the donor site that was covered with allogeneic CDS was more rapid than that covered with FPD. In a representative donor-site wound covered with allogeneic CDS, there was a stratified structure of **epithelial** cells on the underlying connective tissue on day 5, and the **epithelium** had matured by day 12. When covered with FPD a stratified structure of **epithelial** cells was noted on day 8, and the **epithelium** had matured by day 15. We conclude that allogeneic CDS provides a good environment for **epithelialisation**
.

L8 ANSWER 9 OF 30 MEDLINE on STN
AN 2001010003 MEDLINE
TI Evaluation of **dermal-epidermal skin** equivalents ('composite-**skin**') of human keratinocytes in a **collagen**-glycosaminoglycan **matrix**(Integra artificial **skin**).
SO BRITISH JOURNAL OF PLASTIC SURGERY, (2000 Sep) 53 (6) 459-65. Journal code: 2984714R. ISSN: 0007-1226.
AU Kremer M; Lang E; Berger A C
AB Integra artificial **skin** (Integra LifeSciences Corp., Plainsboro, NJ, USA) is a **dermal** template consisting of bovine **collagen**, chondroitin-6-sulphate and a silastic membrane manufactured as Integra. This product has gained widespread use in the clinical treatment of third degree burn wounds and full thickness **skin** defects of different aetiologies. The product was designed to significantly reduce the time needed to achieve final wound closure in the treatment of major burn wounds, to optimise the sparse autologous donor **skin** resources and to improve the durable mechanical quality of the **skin substitute**. The clinical procedure requires two stages. The first step creates a self neodermis, the second creates a self **epidermis** on the neodermis. However, it is desirable to cover major burn wounds early in a single step by a **skin substitute** consisting of a **dermal** equivalent seeded in vitro with autologous keratinocytes ('composite-**skin**') out of which a full thickness **skin** develops in vivo.The goal of this experimental study was to develop a method to integrate human keratinocytes in homogeneous distribution and depth into Integra Artificial **Skin**. The seeded cell-**matrix** composites were grafted onto athymic mice in order to evaluate their potential to reconstitute a human **epidermis** in vivo. We were able to demonstrate that the inoculated human keratinocytes reproducibly displayed a homogeneous pattern of distribution, adherence, proliferation and confluence. The cell-**matrix** composites grafted in this model exhibited good wound adherence, complete healing, minor wound contraction and had the potential to reconstitute an elastic, functional and durable human **skin**. Histologically we were able to show that the inoculated human keratinocytes in vivo colonised the **matrix** in a histomorphologically characteristic **epidermal** pattern (keratomorula, keratinocyte bubbling) and developed a persisting, stratified, keratinising **epidermis** which immunohistologically proved to be of human origin. These experimental results demonstrate the establishment of an effective cell cultivation process which may be suitable for scale-up production of the **epidermal** component as large-scale composite-**skin** grafts. When seeded into Integratrade mark and grafted onto the nude mouse a replacement **skin** with normal functioning **dermal-epidermal** components was developed. These results encourage the design of a clinical trial to assess the function of this composite graft in man.

L8 ANSWER 10 OF 30 MEDLINE on STN
AN 2000456645 MEDLINE
TI Bioartificial skin.
SO CELLS TISSUES ORGANS, (2000) 167 (2-3) 88-94. Ref: 20 Journal code: 100883360. ISSN: 1422-6405.
AU Machens H G; Berger A C; Mailaender P
AB The loss of **skin** has been one of the oldest, yet most frequent and costly problems in our health care system. To restore functional and esthetic integrity in patients with unstable or hypertrophic scars, in burn patients and after **skin** loss for hereditary, traumatic or oncological reasons, an armamentarium of reconstructive surgical procedures including autogenous, allogenous and xenogenous tissue transfer as well as implantation of alloplastic materials has been favored. For several decades there has been increasing interest focused on 'tissue engineering' of **dermal**, **epidermal** and full thickness **skin substitutes** by both biological and synthetic **matrices**. At our institution (Hannover Medical School), a **collagen**/glycosaminoglycan **dermal** regeneration **matrix** has been used for immediate **dermal** coverage after escharectomy in burn injuries as well as for **dermal** replacement

in chronically unstable scars. This article gives an overview on the current state of the art in bioartificial **skin** as well as our personal experience with the **collagen**/glycosaminoglycan **matrix** for **dermal** replacement in different clinical situations.
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L8 ANSWER 11 OF 30 MEDLINE on STN
AN 2000181293 MEDLINE
TI Multistep production of bioengineered skin **substitutes**: sequential modulation of culture conditions.
SO IN VITRO CELLULAR AND DEVELOPMENTAL BIOLOGY. ANIMAL, (2000 Feb) 36 (2) 96-103.
Journal code: 9418515. ISSN: 1071-2690.
AU Auger F A; Pouliot R; Tremblay N; Guignard R; Noel P; Juhasz J; Germain L; Goulet F
AB Many studies are being conducted to define the role of growth factors in cutaneous physiology in order to add cytokines in a timely fashion for optimal tissue engineering of **skin**. This study is aimed at developing a multistep approach for the production of bioengineered **skin substitutes**, taking into account the effects of various growth factors according to the culture time. The use of a serum-supplemented medium throughout the whole culture period of **skin substitutes** was compared to the sequential use of specific additives at defined culture steps. Histological analysis revealed that serum was necessary for keratinocyte proliferation and migration on **dermal substitutes** during the first 2 d after their seeding. However, the serum-free medium presented some advantages when supplemented with different additives at specific culture steps. Interestingly, ascorbic acid added to the **dermal substitutes** before and after keratinocyte seeding maintained their cuboidal morphology in the basal **epidermal** layer. In the absence of serum, **collagen matrix** degradation slowed down, and a better multilayered **epidermal** organization was obtained, notably with retinoic acid. Stratum corneum formation was also enhanced by fatty acids. Thus, sequential addition of exogenous factors to the medium used to produce **skin substitutes** can improve their structural features and functional properties in vitro.

L8 ANSWER 7 OF 30 MEDLINE on STN
AN 2001462033 MEDLINE
TI In vitro characterization of an artificial dermal scaffold.
SO TISSUE ENGINEERING, (2001 Aug) 7 (4) 457-72.
Journal code: 9505538. ISSN: 1076-3279.
AU Ojeh N O; Frame J D; Navsaria H A
AB The treatment of extensive burn injuries has been enhanced by the development of artificial **skin substitutes**. Integra Artificial **Skin**, an acellular **collagen** -glycosaminoglycan (C-GAG) **dermal** equivalent requires a two-stage grafting procedure. However, preseeding the C-GAG **dermal** equivalent with cultured fibroblasts and keratinocytes, with the aim of performing a single-stage grafting procedure, may be beneficial in terms of replacing the requirement for traditional split-**skin** grafts. In this comparative in vitro study, the interactions of cultured human **dermal** fibroblasts and **epidermal** keratinocytes in Integra Artificial **Skin** in comparison to cadaver deepidermalized dermis (DED) was investigated. An increase in cell proliferation and migration in the C-GAG **dermal** equivalent was observed over time. Cocultures of fibroblasts and keratinocytes on both **dermal** equivalents showed positive expression of proliferation, differentiation, and extracellular **matrix** (ECM) protein markers. Organization of keratinocytes in the **epidermal** layers of DED composites were better compared to the C-GAG composites. Deposition of ECM proteins was enhanced in the presence of keratinocytes in both **dermal** equivalents. Results demonstrate that in vitro the C-GAG **dermal** equivalent is biocompatible for cell attachment, migration, proliferation, and differentiation. Preseeding Integra Artificial **Skin** with cultured autologous fibroblasts and keratinocytes for in vivo application, as a single-stage grafting procedure, warrants testing. A better clinical outcome may be achieved as shown by our in vitro results of the coculture composites.

16 ANSWER 2 OF 174 MEDLINE on STN
AN 94159749 MEDLINE
TI A new skin equivalent: keratinocytes proliferated and differentiated on **collagen** sponge containing fibroblasts.
SO PLASTIC AND RECONSTRUCTIVE SURGERY, (1994 Mar) 93 (3) 537-44; discussion 545-6.
Journal code: 1306050. ISSN: 0032-1052.
AU Maruguchi T; Maruguchi Y; Suzuki S; Matsuda K; Toda K; Isshiki N
AB Three types of **artificial skin** containing keratinocytic components were prepared and tested for comparison. Keratinocytes were **cultured** on the **artificial skin dermis** (**collagen** sponge) by the air-liquid interface **culture** method. In order to create continuous keratinocytic **layers** on the **artificial skin dermis**, pores of its uppermost **layer** were filled beforehand with type I **collagen** gel, Matrigel, or fibroblasts. A band of keratinocytes consisting of two to six cell **layers** was formed on **collagen** gel-coated **artificial skin dermis**. On Matrigel-coated **artificial skin dermis**, keratinocytes were piled up into about 20 cell **layers**, but cell differentiation was incomplete; cornified material was not fully developed, and the proportion of cuboidal cells was very high compared with normal **epidermis**. Keratinocytes formed continuous **layers** on the fibroblasts-**artificial skin dermis** complex without gel coating. Keratinocytes proliferated well and differentiated properly on this **matrix**, and their histologic appearance was similar to that of normal **epidermis**. Thus keratinocytes **cultured** on the fibroblast-**artificial skin dermis** complex seem to be a good **skin** equivalent.

L16 ANSWER 3 OF 174 MEDLINE on STN
AN 94190088 MEDLINE
TI Ultrastructural and immunohistochemical characterization of basal cells in three-dimensional culture models of the skin.
SO ARCHIVES OF DERMATOLOGICAL RESEARCH, (1994) 286 (1) 53-61.
Journal code: 8000462. ISSN: 0340-3696.
AU Horiguchi Y; Maruguchi T; Maruguchi Y; Suzuki S; Fine J D; Leigh I M; Yoshiki T; Ueda M; Toda K I; Isshiki N; +
AB Keratinocytes were **cultured** on fibroblast-free **dermal** substitutes made of type I **collagen** film (**collagen dermal** substitute) and an extracellular **matrix** gel film (**matrix dermal** substitute), each of which was laid on a lyophilized type I **collagen** sponge. The morphology of the basal keratinocytes in these three-dimensional **culture** models of the **skin** was studied ultrastructurally and immunohistochemically to assess their differentiation to basal cells. The basal keratinocytes in the **artificial epidermis cultured** on the **collagen dermal** substitute showed poorly organized tonofibril networks and desmosomes. Neither the tonofibril-hemidesmosome complex nor the lamina densa were detected along the interface, where many cytoplasmic projections of basal keratinocytes were noted. There were no detectable antigens of type IV or VII **collagen**, LDA-1, or laminin in the interface. Bullous pemphigoid (BP) and 1-2B7B antigens and integrins were expressed along the cytoplasmic membrane and the projections of the basal keratinocytes. A high molecular weight keratin (keratin 1, 68 kDa, 34 beta B4) was detected only in part of the uppermost **layers** of this **artificial epidermis**. In contrast, basal keratinocytes in the **artificial epidermis** on the **matrix dermal** substitute developed tonofibril networks radiating to desmosomes and hemidesmosomes, under which a primitive lamina densa was present. Basement membrane zone antigens, such as type IV and VII **collagens**, LDA-1 and laminin were noted along the interface as were 1-2B7B and BP antigens and integrins. Laminin and type VII **collagen** were also detected along or in the membrane of the endoplasmic reticulum of basal keratinocytes.(ABSTRACT TRUNCATED AT 250 WORDS)

L16 ANSWER 5 OF 174 MEDLINE on STN
AN 90300729 MEDLINE
TI In vitro effects of **matrix** peptides on a **cultured dermal-epidermal skin** substitute.
SO JOURNAL OF SURGICAL RESEARCH, (1990 Jun) 48 (6) 528-33.
Journal code: 0376340. ISSN: 0022-4804.
AU Cooper M L; Hansbrough J F; Foreman T J
AB Composite **dermal-epidermal skin** substitutes rely on a firm attachment of human keratinocytes (HK) to the **dermal** substrate for **graft** survival on the wound. An in vitro study was performed assessing whether the addition of **matrix** peptides to the **dermal** substrate effected the epithelial thickness. **Cultured grafts** were made by attaching HK to the external surface of a **collagen**-chondroitin 6-sulfate (GAG) membrane and inoculating human fibroblasts (HF) internally. If the **matrix** peptide (RGD Peptide) was added to the **collagen** -GAG membrane prior to placement of the HK and HF, the resultant epithelial **layer** at the end of the normal 4-day **culture** period was significantly thicker (19.7 +/- 0.9 microns versus 13.5 +/- 1.0 microns). Subjectively, the HF content was also greater on the peptide-treated **grafts**. When HF were not placed on the **cultured graft**, i.e., only **collagen**-GAG membrane, RGD peptide, and HK, the resultant epithelial thickness was even greater (28.3 +/- 1.0 microns). These data suggest that addition of **matrix** peptides, which increase cell attachment to the **dermal** substrate, may prove effective in the improvement of this **cultured** composite **dermal-epidermal skin** substitute.

L16 ANSWER 7 OF 174 MEDLINE on STN
AN 2001010003 MEDLINE
TI Evaluation of **dermal-epidermal skin** equivalents ('composite-**skin**') of human keratinocytes in a **collagen**-glycosaminoglycan **matrix**(Integra **artificial skin**).
SO BRITISH JOURNAL OF PLASTIC SURGERY, (2000 Sep) 53 (6) 459-65. Journal code: 2984714R. ISSN: 0007-1226.
AU Kremer M; Lang E; Berger A C
AB Integra **artificial skin** (Integra LifeSciences Corp., Plainsboro, NJ, USA) is a **dermal** template consisting of bovine **collagen**, chondroitin-6-sulphate and a silastic membrane manufactured as Integra. This product has gained widespread use in the clinical treatment of third degree burn wounds and full thickness **skin** defects of different aetiologies. The product was designed to significantly reduce the time needed to achieve final wound closure in the treatment of major burn wounds, to optimise the sparse autologous donor **skin** resources and to improve the durable mechanical quality of the **skin** substitute. The clinical procedure requires two stages. The first step creates a self neodermis, the second creates a self **epidermis** on the neodermis. However, it is desirable to cover major burn wounds early in a single step by a **skin** substitute consisting of a **dermal** equivalent seeded in vitro with autologous keratinocytes ('composite-**skin**') out of which a full thickness **skin** develops in vivo.The goal of this experimental study was to develop a method to integrate human keratinocytes in homogeneous distribution and depth into Integra **Artificial Skin**. The seeded cell-**matrix** composites were grafted onto athymic mice in order to evaluate their potential to reconstitute a human **epidermis** in vivo. We were able to demonstrate that the inoculated human keratinocytes reproducibly displayed a homogeneous pattern of distribution, adherence, proliferation and confluence. The cell-**matrix** composites grafted in this model exhibited good wound adherence, complete healing, minor wound contraction and had the potential to reconstitute an elastic, functional and durable human **skin**. Histologically we were able to show that the inoculated human keratinocytes in vivo colonised the **matrix** in a histomorphologically characteristic **epidermal** pattern (keratomorula, keratinocyte bubbling) and developed a persisting, stratified, keratinising **epidermis** which immunohistologically proved to be of human origin. These experimental results demonstrate the establishment of an effective cell cultivation process which may be suitable for scale-up production of the **epidermal** component as large-scale composite-**skin grafts**. When seeded into Integratrade mark and grafted onto the nude mouse a replacement **skin** with normal functioning **dermal-epidermal** components was developed. These results encourage the design of a clinical trial to assess the function of this composite **graft** in man.

L16 ANSWER 11 OF 174 MEDLINE on STN
AN 2000269870 MEDLINE
TI A new skin equivalent model: dermal substrate that combines de-epidermized dermis with fibroblast-populated **collagen matrix**.
SO JOURNAL OF DERMATOLOGICAL SCIENCE, (2000 Jun) 23 (2) 132-7.
Journal code: 9011485. ISSN: 0923-1811.
AU Lee D Y; Ahn H T; Cho K H
AB **Epidermis** reconstructed on de-**epidermized** **dermis** (RE-DED) and on fibroblast-populated **collagen** **matrix** (Living **Skin** Equivalent) showed a histologic resemblance to native **epidermis**. However, some abnormalities have been found including different expression pattern of differentiation markers from native **epidermis**. In this study, to reconstruct an **epidermis** model resembling native **epidermis** more closely than previous **skin** equivalents, de-**epidermized** **dermis** (DED) was raised on fibroblast-populated **collagen** **matrix** and keratinocytes were **cultured** on top of the DED at the air-liquid interface. The new **skin** equivalent like RE-DED showed a similar morphology to that of native **epidermis**. Immunohistochemical studies revealed that differentiation markers such as involucrin, loricrin and filaggrin but not keratin 1 expressed similar pattern characteristics to native **epidermis** compared with those of RE-DED. In addition, the new model showed some fibroblasts in the DED as a result of migration from the fibroblast-populated **collagen** **matrix**, mimicking a living **dermis** in vivo. These results indicate that the new model seems to be a better **skin** equivalent model than previous models. Also, they provide additional evidence that the presence of fibroblasts improves **epidermal** differentiation.

L16 ANSWER 15 OF 174 MEDLINE on STN
AN 1999363750 MEDLINE
TI Comparison of cultured and uncultured keratinocytes seeded into a **collagen**-GAG **matrix** for skin replacements.
SO BRITISH JOURNAL OF PLASTIC SURGERY, (1999 Mar) 52 (2) 127-32.
Journal code: 2984714R. ISSN: 0007-1226.
AU Butler C E; Yannas I V; Compton C C; Correia C A; Orgill D P
AB A well-characterised **collagen**-glycosaminoglycan (CG) **matrix** functions as an extracellular **matrix** analogue (ECMA) of **dermis** on full-thickness wounds. The **epidermis** can be reconstituted by seeding autologous uncultured keratinocytes into the **matrix** prior to grafting. We hypothesised that seeding the CG **matrix** with keratinocytes **cultured** to sub-confluence may provide the ECMA with more proliferating keratinocytes than with uncultured keratinocytes. Autologous cells were isolated from split-thickness **skin grafts** and **cultured** to sub-confluence. ECMAs were seeded by centrifuging **cultured** (n = 8) or uncultured (n = 8) autologous keratinocytes into a CG **matrix** at a density of 100,000 cells/cm2, then applied onto full-thickness wounds on Yorkshire pigs. Gross and histologic observations were made up to 21 days post-grafting. At 14 days, a fully differentiated **epidermis** was present on all **graft** sites, but the **epidermis** of the **cultured**-cell-seeded matrices was thicker, 180 (19) microns, than the uncultured-cell-seeded matrices, 110 (18) microns. The **epidermis** of **cultured**-cell-seeded matrices was acanthotic, containing 14 (4) cell **layers**, as compared to uncultured-cell-seeded matrices, 9 (1) cell **layers**. The number of subepithelial keratinocyte cysts/cm cross-section present in the neodermis was also greater in **cultured**-, 1.35 (0.37), than in uncultured-cell-seeded matrices, 0.47 (0.35). **Epidermal** confluence on day 14 was 96 (3)% on **cultured**-cell-seeded **grafts** and 50 (17)% on uncultured-cell-seeded **grafts**. These results are consistent with the hypothesis that the process of in vitro cell cultivation increases the proportion of dividing cells in preference to differentiated cells. This technology may be useful in reconstruction of specialised bilayer tissues with minimal donor sites.

L20 ANSWER 1 OF 62 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1986:597218 CAPLUS
DN 105:197218
TI A man-made skin composed of two **layers**: **collagen** and a poly-.alpha.-amino acid
SO Eur. Pat. Appl., 13 pp.
CODEN: EPXXDW
IN Kuroyanagi, Yoshimitsu; Miyata, Teruo; Seno, Manabu
AB A double-**layered artificial skin** is prepd. by laminating a **collagen** sponge sheet and a poly-.alpha.-amino acid membrane that has a good affinity for tissue cells and an appropriate permeability to moisture. When the **skin** is applied to burns, cuts or wounds, the fibroblasts proliferate in the **collagen** sponge sheet forming a three-dimensional structure, while the **epidermal** cells proliferate in the region between the poly-.alpha.-amino acid membrane and a **collagen** sponge sheet. The poly-.alpha.-amino acid membrane plays a role in protecting affected part and in providing an optimum condition for the proliferation of fibroblasts and **epidermal** cells, and then it falls off as the **epidermis** completely regenerates. On the other hand, the **collagen** sponge sheet assimilates in the living tissue after having played a **general** role of the **dermis**.

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 167828	A1	19860115	EP 1985-106946	19850604
	EP 167828	B1	19900314		
	R: BE, CH, DE, FR, GB, IT, LI, NL, SE				
	JP 60261460	A2	19851224	JP 1984-118276	19840611
	JP 63017458	B4	19880413		

L20 ANSWER 8 OF 62 MEDLINE on STN
AN 1998184226 MEDLINE
TI **Genetically** modified human keratinocytes overexpressing PDGF-A enhance the performance of a composite skin graft.
SO HUMAN GENE THERAPY, (1998 Mar 1) 9 (4) 529-39.
Journal code: 9008950. ISSN: 1043-0342.
AU Eming S A; Medalie D A; Tompkins R G; Yarmush M L; Morgan J R
AB **Skin** loss due to burns and ulcers is a major medical problem. Bioengineered **skin** substitutes that use **cultured** keratinocytes as an **epidermal layer** with or without analogues of the **dermis** are one strategy for **skin** repair. However, none can achieve definitive wound closure, function, or cosmesis comparable to split-thickness autografts. Moreover, autograft donor sites, which require time to heal, may be limited or have attendant problems such as infection or functional/cosmetic deficiencies. To determine if the performance of composite **skin grafts** of keratinocytes on a **dermal** analogue could be enhanced, human keratinocytes were **genetically** modified to overexpress platelet-derived growth factor A chain (PDGF-A). Composite **grafts** of modified keratinocytes seeded onto acellular **dermis**, prepared from cryopreserved cadaver **skin**, secreted PDGF-AA protein in vitro [90 ng/**graft** (1.5 x 1.5 cm)/24 hr]. To test their performance in a wound healing model, composite **grafts** were transplanted to full-thickness excisional wounds on the back of athymic mice. PDGF-A **grafts** formed a stratified differentiated **epidermis** similar to control **grafts**. The acellular **dermis** was repopulated with host fibrovascular cells and by day 7, the PDGF-A **grafts** had significantly more cells in the **dermis** and increased staining for murine **collagen** types I and IV. At this early time point, wound contraction was also significantly inhibited in PDGF-A **grafts** versus control **grafts**. Thus, PDGF-A overexpression improves **graft** performance during the first critical week after transplantation.

L20 ANSWER 12 OF 62 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2003:717667 CAPLUS
DN 139:235491
TI Surgical device for skin therapy
SO U.S. Pat. Appl. Publ., 8 pp.
CODEN: USXXCO
IN Boyce, Steven T.
AB A device, and method of making the device, capable of therapeutic treatment and/or for in vitro testing of human **skin**. The device may be used on **skin** wounds for burned, injured, or diseased **skin**, and provides structures and functions as in normal uninjured **skin**, such as barrier function, which is a definitive property of normal **skin**. The device contains **cultured dermal** and **epidermal** cells on a biocompatible, biodegradable reticulated **matrix**. All or part of the cells may be autologous, from the recipient of the **cultured skin** device, which advantageously eliminates concerns of tissue compatibility. The cells may also be modified **genetically** to provide 1 or more factors to facilitate healing of the engrafted **skin** replacement, such as an angiogenic factor to stimulate growth of blood vessels. The inventive device is easy to handle and manipulate for surgical transplant, can be made into large sheets to minimize the no. of **grafts** required to cover a large surface area to be treated, and can be produced within the time frame to treat a burned individual requiring a **skin graft**.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 2003170892	A1	20030911	US 2002-92237	20020306
WO 2003076604	A2	20030918	WO 2003-US6584	20030303

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

L20 ANSWER 56 OF 62 MEDLINE on STN
AN 96215727 MEDLINE
TI Permanent skin replacement using chimeric epithelial cultured sheets comprising xenogeneic and syngeneic keratinocytes.
SO TRANSPLANTATION, (1996 May 15) 61 (9) 1290-300.
Journal code: 0132144. ISSN: 0041-1337.
AU Rouabhia M
AB The present study was undertaken to evaluate the possibility of permanent **skin** replacement using chimeric xenogeneic-syngeneic graftable sheets previously obtained in vitro. Newborn (<3 days old) BALB/c and human keratinocytes were isolated and cocultured in different ratios as follows: 50% BALB/c to 50% human and 25% BALB/c to 75% human keratinocytes. Four to 5 days after **culture** and prior to their grafting, all chimeric sheets contained both cell types in ratios similar to those used to seed the initial chimeric **cultures**. Fourteen and 30 days after chimeric sheet grafting onto BALB/c mice dorsum, the newly **generated** cutaneous tissue showed a histologically well-organized **epidermis** presenting basal and suprabasal cell **layers**. Cutaneous cells in these structures secreted laminin and type IV **collagen** in blood vessels, and at ground level of the **dermoepidermal** junction there were signs of physiologically active **skin**. Cell phenotyping revealed the presence of only syngeneic keratinocytes, whereas xenogeneic cells were passively eliminated without a total rejection of the chimeric implant. This selective and passive elimination of xenogeneic keratinocytes went through cellular and humoral immunity activation. Data suggest that this chimeric **culture** method can be used for cutaneous therapies such as large congenital nevi, **skin** ulcers, and extensively burned **skin**. Indeed, for large third-degree wounded **skin** treatment, this **culture** method may shorten the time (4-5 weeks) needed for cell growth and graftable sheet production. Moreover, since the ultimate aim in allogeneic and xenogeneic transplantation is to achieve an immunological acceptance and tolerance to these foreign tissues, the chimeric **culture** approach may provide ways to lighten tolerance phenomena on cutaneous tissue.

L Number	Hits	Search Text	DB	Time stamp
-	2	("5711172").PN.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/07/08 10:50
-	2	("5976878").PN.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/07/08 10:47
-	2	("5273900").PN.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/07/08 10:53
-	8	boyce NEAR steven	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/07/08 10:53
-	7	(US-4673649-$ or US-6057148-$ or US-6043089-$).did. or (WO-8808305-$).did. or (US-5976878-$ or US-5711172-$ or US-5273900-$).did.	USPAT; EPO; DERWENT	2003/07/08 10:55
-	45	Naughton NEAR Gail	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/10/09 11:47
-	29	(US-5976878-$ or US-5273900-$ or US-6043089-$ or US-6057148-$ or US-4673649-$ or US-5858721-$ or US-5830708-$ or US-5785964-$ or US-5624840-$ or US-5580781-$ or US-5578485-$ or US-5541107-$ or US-5518915-$ or US-5160490-$ or US-5516681-$ or US-5516680-$ or US-5512475-$ or US-5510254-$ or US-5460939-$ or US-5443950-$ or US-5266480-$ or US-5032508-$ or US-4963489-$).did. or (WO-8808305-$ or WO-9943787-$ or EP-358506-$).did. or (JP-2000189158-$ or JP-2001258555-$).did. or (US-5711172-$).did.	USPAT; EPO; JPO; DERWENT	2003/10/14 12:00
-	22	((US-5976878-$ or US-5273900-$ or US-6043089-$ or US-6057148-$ or US-4673649-$ or US-5858721-$ or US-5830708-$ or US-5785964-$ or US-5624840-$ or US-5580781-$ or US-5578485-$ or US-5541107-$ or US-5518915-$ or US-5160490-$ or US-5516681-$ or US-5516680-$ or US-5512475-$ or US-5510254-$ or US-5460939-$ or US-5443950-$ or US-5266480-$ or US-5032508-$ or US-4963489-$).did. or (WO-8808305-$ or WO-9943787-$ or EP-358506-$).did. or (JP-2000189158-$ or JP-2001258555-$).did. or (US-5711172-$).did.) and (insulin vitamin strontium)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/10/14 12:01
-	1	((US-5976878-$ or US-5273900-$ or US-6043089-$ or US-6057148-$ or US-4673649-$ or US-5858721-$ or US-5830708-$ or US-5785964-$ or US-5624840-$ or US-5580781-$ or US-5578485-$ or US-5541107-$ or US-5518915-$ or US-5160490-$ or US-5516681-$ or US-5516680-$ or US-5512475-$ or US-5510254-$ or US-5460939-$ or US-5443950-$ or US-5266480-$ or US-5032508-$ or US-4963489-$).did. or (WO-8808305-$ or WO-9943787-$ or EP-358506-$).did. or (JP-2000189158-$ or JP-2001258555-$).did. or (US-5711172-$).did.) and (vitamin strontium)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/10/14 12:02

-	2	((US-5976878-$ or US-5273900-$ or US-6043089-$ or US-6057148-$ or US-4673649-$ or US-5858721-$ or US-5830708-$ or US-5785964-$ or US-5624840-$ or US-5580781-$ or US-5578485-$ or US-5541107-$ or US-5518915-$ or US-5160490-$ or US-5516681-$ or US-5516680-$ or US-5512475-$ or US-5510254-$ or US-5460939-$ or US-5443950-$ or US-5266480-$ or US-5032508-$ or US-4963489-$).did. or (WO-8808305-$ or WO-9943787-$ or EP-358506-$).did. or (JP-2000189158-$ or JP-2001258555-$).did. or (US-5711172-$).did.) and triiodothy$9	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/10/14 12:11
-	0	((US-5976878-$ or US-5273900-$ or US-6043089-$ or US-6057148-$ or US-4673649-$ or US-5858721-$ or US-5830708-$ or US-5785964-$ or US-5624840-$ or US-5580781-$ or US-5578485-$ or US-5541107-$ or US-5518915-$ or US-5160490-$ or US-5516681-$ or US-5516680-$ or US-5512475-$ or US-5510254-$ or US-5460939-$ or US-5443950-$ or US-5266480-$ or US-5032508-$ or US-4963489-$).did. or (WO-8808305-$ or WO-9943787-$ or EP-358506-$).did. or (JP-2000189158-$ or JP-2001258555-$).did. or (US-5711172-$).did.) and strontium	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/10/14 12:11
-	150	skin and (strontium NEAR chloride)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/10/14 12:12
-	3	((skin and (strontium NEAR chloride)) and insulin) and triiodo$9	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/10/14 12:12
-	40	(skin and (strontium NEAR chloride)) and insulin	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/10/14 12:17
-	1	UCMC NEAR "161" NEAR medium	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/10/14 12:17
-	1	UCMC WITH medium	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/10/14 12:18